Draft NTP Technical Report TR584
on
Indole-3-Carbinol
(Gavage Studies)

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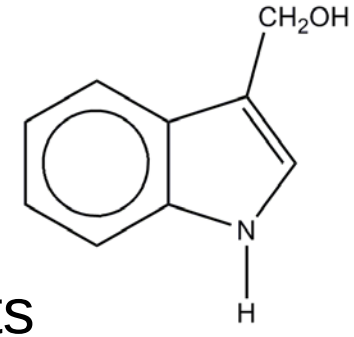
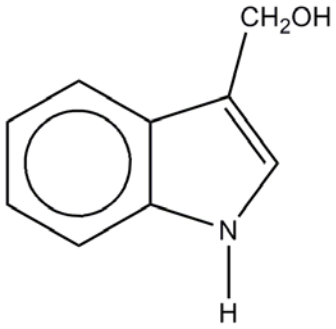
Background



- Nominated by the National Cancer Institute (NCI) based on its occurrence in natural products and its potential use as a cancer chemopreventive agent
- Derived from cruciferous vegetables of the *Brassica* genus (Brussels sprouts, cauliflower, cabbage, kale, turnips, and broccoli)
- Commercially-available dietary supplements
- Dietary supplements containing indole-3-carbinol are regulated under the Dietary Supplement Health and Education Act (1994)

Toxicology



- Indole-3-carbinol activates the Ah Receptor, a mechanism often associated with toxicity and carcinogenicity
  - Induction of cytochrome P450s
- Tumor promoter in multiple organs in rodent and trout models
- Chemoprotective in a number of animal models
  - Block initiation, inhibit formation carcinogen–DNA adducts, and alter cell cycle progression, proliferation, and apoptosis

NTP Program of Study for Indole-3-Carbinol

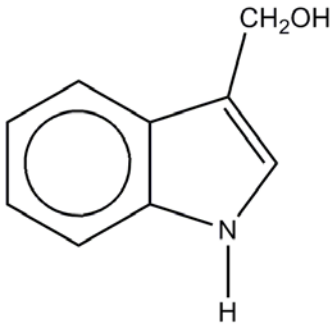
- **3-Month** gavage studies in F344/N rats and B6C3F1/N mice
- **2-Year** gavage studies in Harlan Sprague Dawley rats and B6C3F1/N mice
  - 1-week and 4-week interim time points to further evaluate lymphatic ectasia observed in 3-month studies
  - Hepatic gene expression evaluation (3-month interim)
- **Genotoxicity** testing
  - *Salmonella*, *E. coli.*, micronucleus

Genetic Toxicity Results for Indole-3-Carbinol

Test	Results
Bacterial Mutagenicity (*Overall results across 3 independent studies*)	***Weak* positive** in *S. typhimurium* TA100 (+/- S9) **Equivocal** in TA97 (+/- S9) and *E. coli WP2 uvrA*$^{-/-}$ (- S9) **Negative** in TA98, TA1535, TA1537, TA102, TA104 (+/- S9)
Mouse peripheral blood micronucleus	**Negative** in males and females
Rat bone marrow micronucleus	**Negative** in males (Females not tested)

3-Month Toxicology Studies

- Male and female F344/N rats and B6C3F1/N mice (n=10)
- Administered Doses (5 days/week)
  - Rats: 0, 18.75, 37.5, 75, 150, and 300 mg/kg in corn oil
  - Mice: 0, 15.6, 31.25, 62.5, 125, and 250 mg/kg in corn oil



3-Month Results in F344/N Rats

- No significant treatment-related effects were observed on survival, clinical chemistry, hematology, or gross lesions
- Significant decrease (12%) in body weight gain in 300 mg/kg males compared to controls
- Dose-dependent increases in absolute and relative liver weights at all doses in both sexes
  - No corresponding change in the incidences of hepatocellular hypertrophy
- Increases kidney weights in males at ≥75 mg/kg, and in all doses in females
- Dose-dependent increase in hepatic CYP 1A1 and 1A2 and pulmonary 1A1 activity
  - 80- to 100-fold increase in hepatic CYP1A1 activity in males and females, respectively
  - Approximately 5-fold increase in hepatic CYP1A2 activity in males and females

3-Month Results in F344/N Rats

- Treatment-related increase in the incidence of lymphatic ectasia of the jejunum, duodenum, and mesenteric lymph nodes
  - Accumulation of macrophages within the intestinal lamina propria and in the lymph node subcapsular sinuses
  - Specialized staining with Oil Red O and Sudan Black confirmed the presence of lipid in the lymphatic

3-Month Results in F344/N Rats

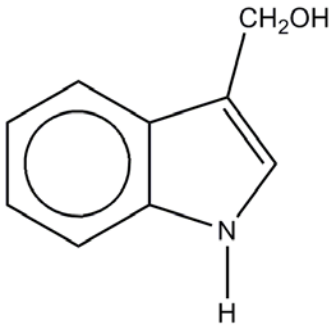
- Increased estrous cycle length (approximately 1 day) in 300 mg/kg females
  - Probability of extended diestrus was significantly higher in the 300 mg/kg group*
- Selected doses of 0, 75, 150, and 300 mg/kg for chronic gavage studies in Harlan Sprague Dawley rats

*Markov transition matrix analyses of estrous cyclicity

3-Month Results in Mice

- No significant treatment-related effects were observed on body weight, survival, hematology, or gross lesions
- Treatment-related increases in liver weights at ≥125 mg/kg in males and at all doses in females
  - No corresponding change in the incidences of hepatocellular hypertrophy
- Treatment-related increase in hepatic CYP 1A1 and 1A2 and pulmonary 1A1 activity in both sexes
- Decreased sperm motility at all doses
- Increased probability of extended diestrus in 250 mg/kg females
- Selected doses of 0, 62.5, 125, and 250 mg/kg for chronic gavage studies in B6C3F1/N mice

Chronic Rat Studies



- No treatment-related effects on body weight, survival, or clinical signs

Incidences of Lesions in the Uterus

Standard Evaluation	0 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Adenocarcinoma	0*	1	4	4
Endometrium, squamous metaplasia	12 (1.9)	18 (2.2)	20* (2.1)	11 (1.8)

Number of animals with lesion (average severity grade of lesion:1=minimal, 2=mild, 3=moderate, 4=marked)
* P < 0.05; ** P < 0.01 by Poly-3 test

- Uterine masses observed grossly at necropsy likely related to the increased incidences of uterine adenocarcinoma
- Positive trend, increased in all dose groups
- Based on the occurrence of adenocarcinomas, a more comprehensive evaluation of the uteri were conducted

Incidences of Lesions in the Uterus

Standard Evaluation	0 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Adenocarcinoma	0*	1	4	4
Endometrium, squamous metaplasia	12 (1.9)	18 (2.2)	20* (2.1)	11 (1.8)

Extended Evaluation	0 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Adenocarcinoma	5	3	10	8
Adenocarcinoma (multiple)	1	0	3	2
Adenosquamous carcinoma	0	0	1	0
Adenoma	0	2	1	1
Adenocarcinoma or Adenoma	5	5	11	9

Number of animals with lesion (average severity grade of lesion:1=minimal, 2=mild, 3=moderate, 4=marked)
* P < 0.05; ** P < 0.01 by Poly-3 test

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Extended Evaluation	0 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Adenocarcinoma (includes multiples)	5	3	10	8
Adenocarcinoma (multiple)	1	0	3	2
Adenosquamous carcinoma	0	0	1	0
Adenoma	0	2	1	1
Adenocarcinoma or Adenoma	5	5	11	9
Adenocarcinoma and Adenosquamous carcinoma	5	4	13*	10
Adenoma, Adenosquamous carcinoma, or Adenocarcinoma	5	5	14*	11

- Considered **some evidence** for carcinogenicity

Incidences of Neoplasms in Female Rat Skin

Skin	0 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Fibroma	1	0	0	4
Fibrosarcoma	1	0	0	1
Fibroma or fibrosarcoma	2*	0	0	5

* $P < 0.05$ by Poly-3 test

- Positive statistical trend, not significantly increased at 300 mg/kg
- Considered an equivocal effect that may have been related to treatment

Nonneoplastic Lesions in Male Rats

Small Intestine	0 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Duodenum, Lymphatic ectasia	0	0	15** (1.5)	14** (1.4)
Jejunum, Lymphatic ectasia	0	2 (1.0)	27** (1.7)	41** (2.0)
Mesenteric Lymph Node				
Lymphatic ectasia	0	0	1 (3.0)	5* (1.4)
Liver				
Bile duct cyst	0	0	2 (2.0)	5* (2.4)
Thyroid Gland				
Follicular cell hypertrophy	21 (1.8)	34** (1.9)	33** (2.1)	36** (2.6)

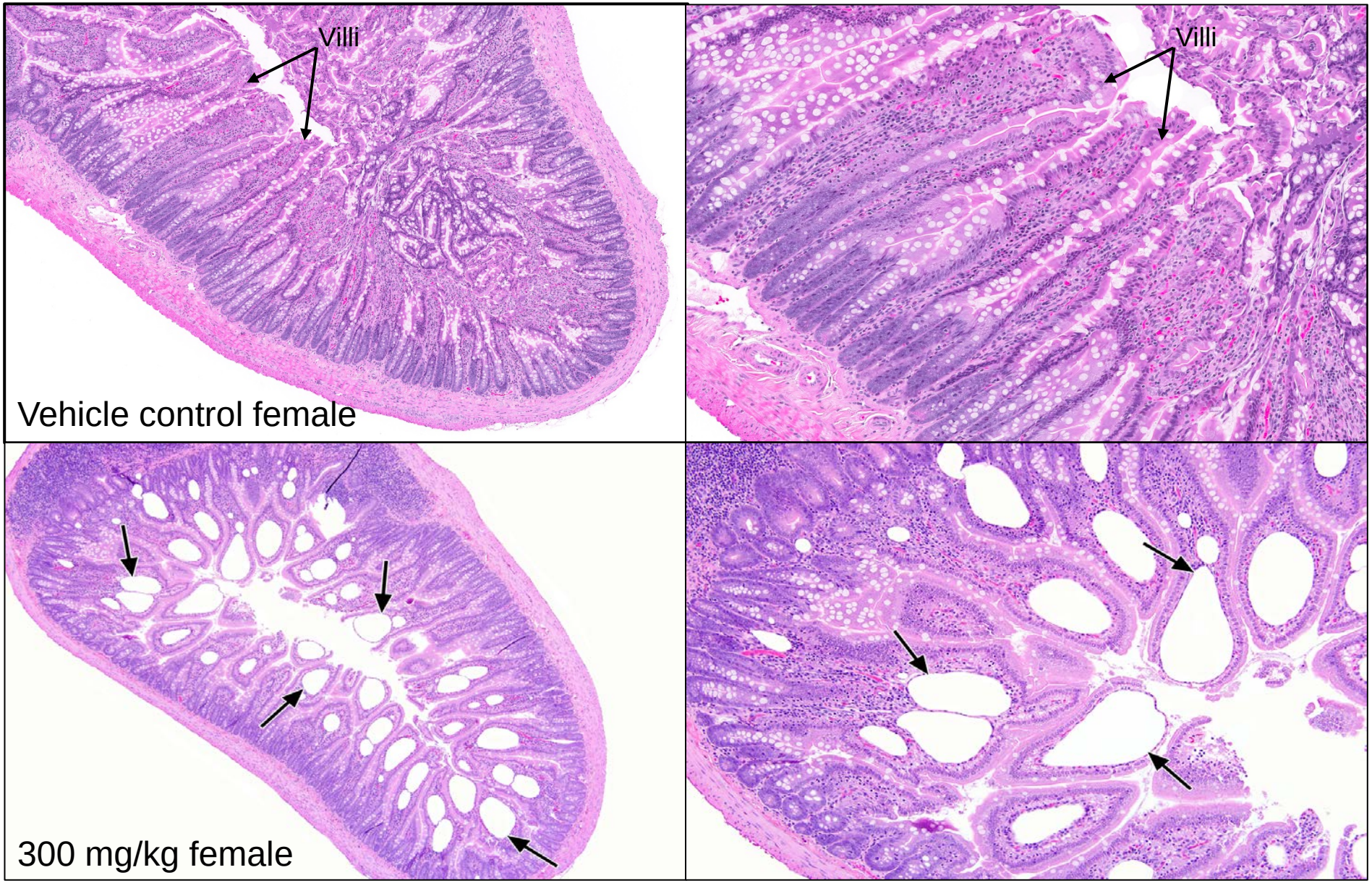
Number of animals with lesion (average severity grade of lesion:1=minimal, 2=mild, 3=moderate, 4=marked)
* P < 0.05; ** P < 0.01 by Poly-3 test

Nonneoplastic Lesions in Female Rats

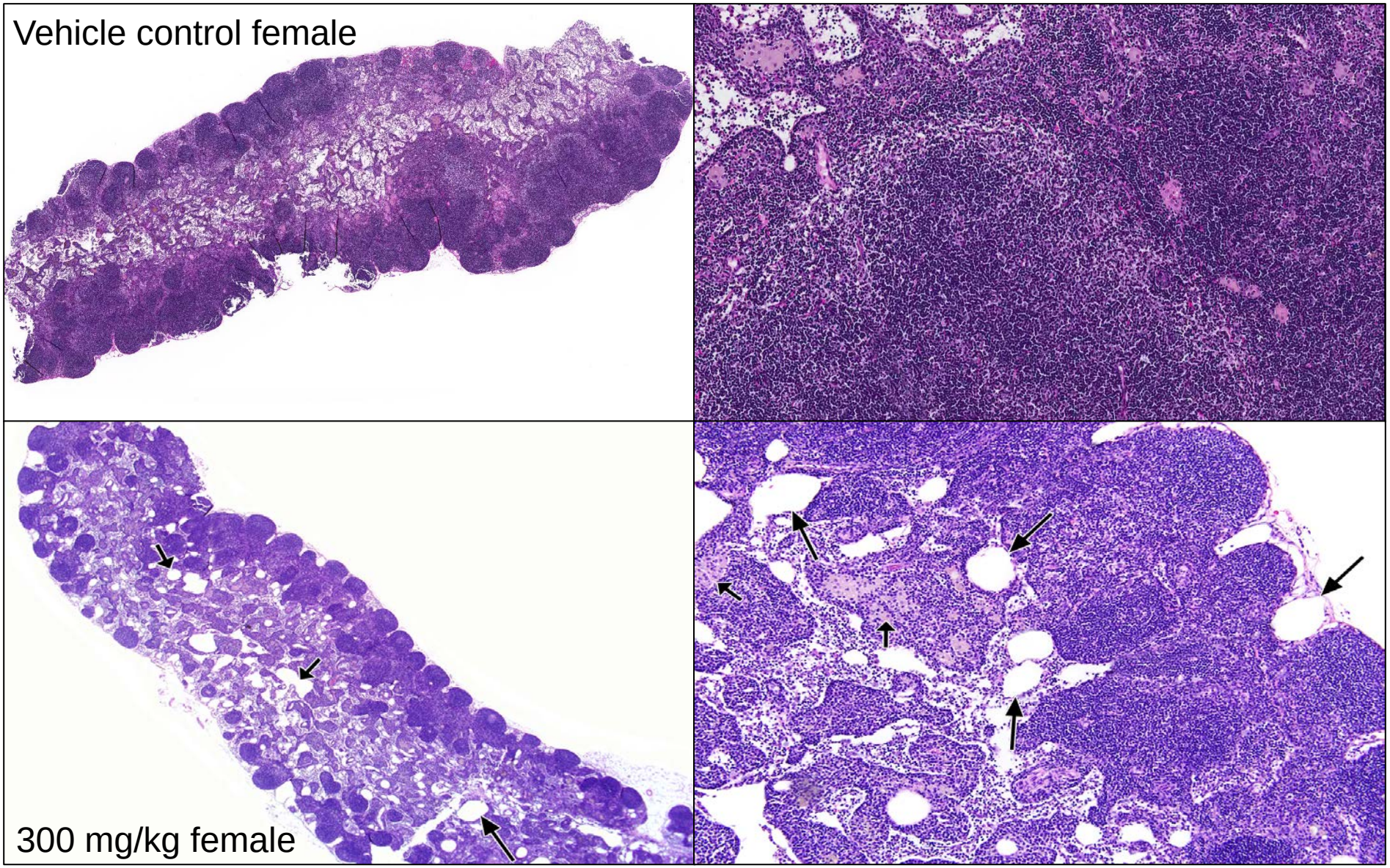
Small Intestine	0 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Duodenum, Lymphatic ectasia	0	0	16** (1.2)	38** (1.5)
Jejunum, Lymphatic ectasia	0	0	30** (1.7)	47** (2.5)
Mesenteric Lymph Node				
Lymphatic ectasia	0	0	1 (1.0)	15** (1.7)
Liver				
Clear cell foci	6	7	4	18**
Eosinophilic foci	0	4	5*	6*

Number of animals with lesion (average severity grade of lesion:1=minimal, 2=mild, 3=moderate, 4=marked)
* P < 0.05; ** P < 0.01 by Poly-3 test

Lymphatic Ectasia in the Small Intestine



Lymphatic Ectasia in the Mesenteric Lymph Node



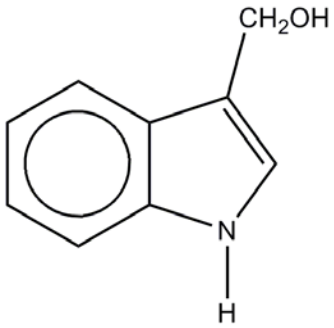
Hepatic Gene Expression Results

- 3-Month interim evaluation
  - Female control and 300 mg/kg (n=5)
- Activation of multiple xenobiotic transcription factors
  - Pronounced activation of AhR and Nrf2
- Upregulation of genes associated with xenobiotic metabolism
  - Cyp1a1 (334-fold)
  - Cyp1b1 (75-fold)
  - Cyp2b1/Cyp2b2 (32-fold)

Evidence of Carcinogenic Activity in Rats

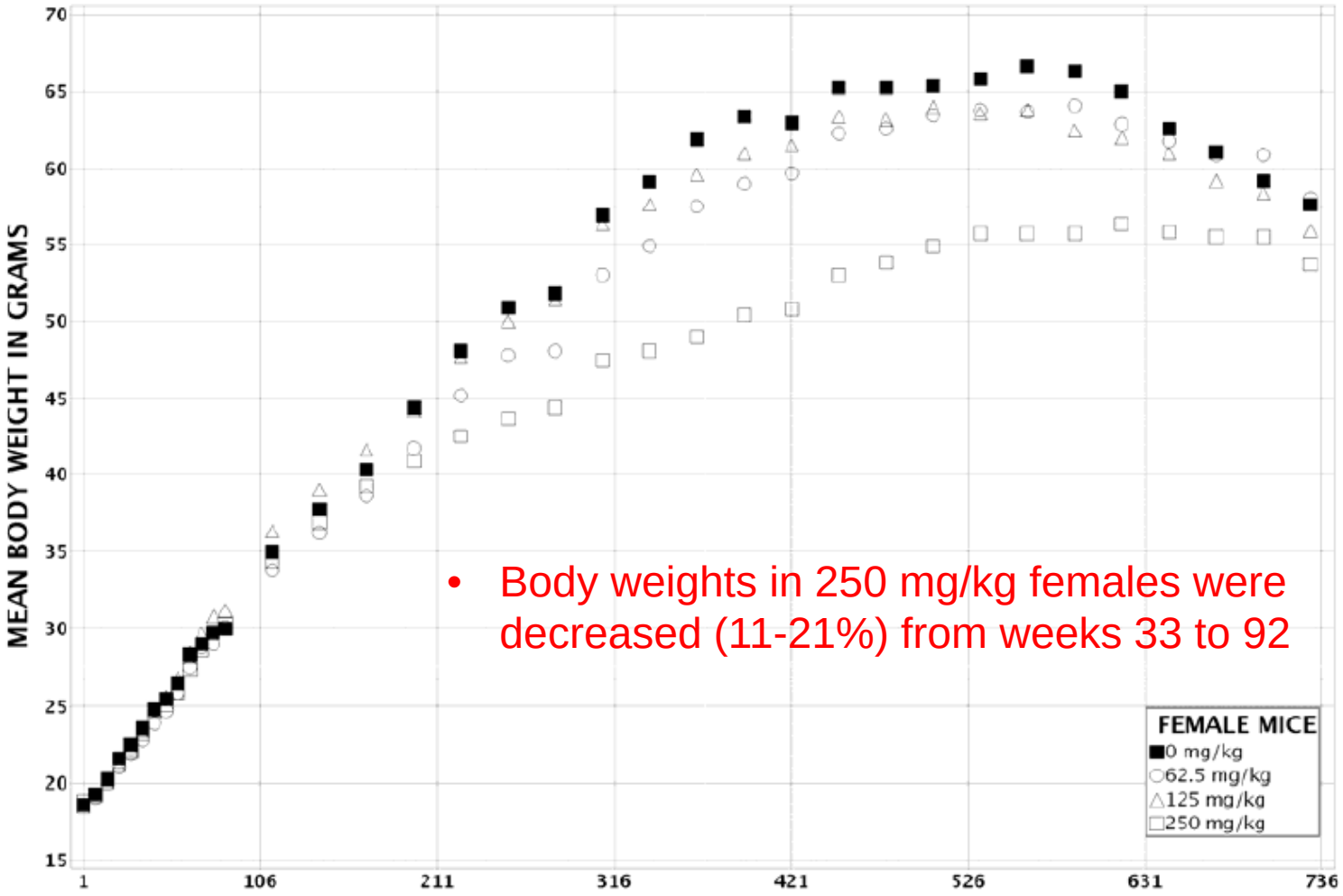
- **Males**
  - *No evidence* in male Harlan Sprague Dawley rats administered 75, 150, or 300 mg/kg indole-3-carbinol
- **Females**
  - *Some evidence* in female rats based on increased incidences of malignant uterine neoplasms (primarily adenocarcinoma)
  - Occurrences of fibroma and sarcoma of the skin may have been related to indole-3-carbinol administration

Chronic Mouse Studies



- No treatment-related effects on survival or clinical signs

Female Mouse Body Weights



- Body weights in 250 mg/kg females were decreased (11-21%) from weeks 33 to 92

Chronic Mouse Study – Histopathology

- **Liver**
  - Neoplastic (males only): Hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma
  - Nonneoplastic: Clear cell foci (males), eosinophilic foci (females)
- **Glandular Stomach**
  - Epithelial hyperplasia, chronic inflammation, pigmentation (males and females)
- **Nose**
  - Nerve atrophy; olfactory epithelium respiratory metaplasia; olfactory epithelium atrophy; respiratory epithelium hyperplasia (males and females)
  - Olfactory epithelium necrosis; respiratory epithelium hyaline droplet accumulation (males only)
  - Inflammation (females only)

Liver Neoplasms in Male Mice

Lesions	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
Hepatocellular Adenoma (includes multiples)[a]	26**	32	31	41**
Hepatocellular Carcinoma (includes multiples)[b]	12	11	29**	14
Hepatoblastoma (includes multiples)[c]	3**	4	4	14**

* P < 0.05; ** P < 0.01 (n=50)

[a] Historical incidence: 145/250 (range of 52-64%) for corn oil vehicle; 594/949 (range of 48-78%) for all routes

[b] Historical incidence: 87/250 (range of 22-44%) for corn oil vehicle; 348/949 (range of 22-56%) for all routes

[c] Historical incidence: 9/250 (range of 0-6%) for corn oil vehicle; 40/949 (range of 0-12%) for all routes

- Significant trend for adenoma and hepatoblastoma, pairwise increase in individual lesions at 125 or 250 mg/kg

Liver Neoplasms in Male Mice

Lesions	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
Hepatocellular Adenoma (includes multiples)	26*	32	31	41**
Multiple Adenoma	15	25*	16	33*
Hepatocellular Carcinoma (includes multiples)	12	11	29**	14
Multiple Carcinoma	5	3	18**	8
Hepatoblastoma (includes multiples)	3**	4	4	14**
Multiple Hepatoblastoma	0	1	3	7*

* P < 0.05; ** P < 0.01 (n=50)

[a] Historical incidence: 145/250 (range of 52-64%) for corn oil vehicle; 594/949 (range of 48-78%) for all routes

[b] Historical incidence: 87/250 (range of 22-44%) for corn oil vehicle; 348/949 (range of 22-56%) for all routes

[c] Historical incidence: 9/250 (range of 0-6%) for corn oil vehicle; 40/949 (range of 0-12%) for all routes

- Significant trend for adenoma and hepatoblastoma, pairwise increase in individual lesions at 125 or 250 mg/kg
- Increased multiples

Liver Neoplasms in Male Mice

Lesions	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
Hepatocellular Adenoma	26*	32	31	41**
Hepatocellular Carcinoma	12	11	29**	14
Hepatoblastoma	3**	4	4	14**
Combined Lesions				
Hepatocellular adenoma or carcinoma[a]	35*	36	43	44*
Hepatocellular carcinoma or hepatoblastoma[b]	15**	13	30**	25*
Hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma[c]	36**	36	44*	45*

* P < 0.05; ** P < 0.01 (n=50)

[a] Historical incidence:189/250 (range of 70-78%) for corn oil vehicle; 742/949 (range of 64-90%) for all routes

[b] Historical incidence: 93/250 (range of 24-48%) for corn oil vehicle; 371/949 (range of 22-58%) for all routes

[c] Historical incidence: 190/250 (range of 72-78%) for corn oil vehicle; 746/949 (range of 64-90%) for all routes

- Considered **clear evidence** for carcinogenicity

Nonneoplastic Lesions of the Glandular Stomach

Males	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
Epithelium Hyperplasia	0	1 (1.0)	22 (1.2)**	40 (1.5)**
Chronic inflammation	1 (1.0)	1 (1.0)	18 (1.0)**	45 (1.0)**
Pigmentation	0	1 (1.0)	38 (1.0)**	48 (1.1)**
Females	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
Epithelium Hyperplasia	1 (2.0)	7 (1.3)*	10 (1.2)**	35 (1.4)**
Chronic inflammation	0	15 (1.0)**	29 (1.1)**	47 (1.3)**
Pigmentation	0	15 (1.0)**	31 (1.2)**	49 (1.9)**

Number of animals with lesion (average severity grade of lesion:1=minimal, 2=mild, 3=moderate, 4=marked)
* P < 0.05; ** P < 0.01 by Poly-3 test

Nonneoplastic Lesions in the Nose

Males	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
Nerve atrophy	0	0	0	8 (2.0)**
Olfactory epithelium, Respiratory Metaplasia	14 (1.1)	14 (1.3)	20 (1.5)	27 (1.4)*
Olfactory epithelium, Atrophy	3 (2.0)	5 (1.6)	11 (1.4)*	17 (1.5)**
Olfactory epithelium, Necrosis	0	0	0	6 (2.2)*
Respiratory epithelium, Hyaline droplet accumulation	18 (1.2)	34 (1.1)**	30 (1.1)*	26 (1.2)
Respiratory epithelium, Hyperplasia	35 (1.0)	40 (1.2)	41 (1.2)	45 (1.3)*
Females				
Nerve atrophy	0	0	1 (2.0)	50 (3.0)**
Olfactory epithelium, Respiratory Metaplasia	7 (1.0)	8 (1.0)	16 (1.0)*	49 (2.9)**
Olfactory epithelium, Atrophy	1 (1.0)	2 (1.0)	3 (2.0)	45 (2.0)**
Respiratory epithelium, Hyperplasia	32 (1.0)	31 (1.0)	38 (1.0)	50 (3.0)**
Inflammation	4 (1.5)	1 (1.0)	8 (1.1)	39 (1.2)**

Number of animals with lesion (average severity grade of lesion:1=minimal, 2=mild, 3=moderate, 4=marked)
* P < 0.05; ** p < 0.01 by Poly-3 test

Evidence of Carcinogenic Activity in Mice

- **Males**
  - *Clear evidence* in male B6C3F1/N mice based on increased incidences of liver neoplasms (hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma)
- **Females**
  - *No evidence* in female B6C3F1/N mice administered 0, 62.5, 125, or 250 mg/kg